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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	David Moore et al.	Art Unit:	1646
Serial No.:	09/365,576	Examiner:	M. Pak
Filed:	August 2, 1999	Customer No.:	21559
Title:	RETINOID X RECEPTOR-INTERACTING POLYPEPTIDES AND RELATED MOLECULES AND METHODS		

Mail Stop Appeal
Commissioner for Patents
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Alexandria, VA 22313-1450

APPEAL BRIEF UNDER 37 C.F.R. § 41.37

In support of Appellant's Notice of Appeal that was filed in connection with the above-captioned case on April 8, 2005, and with reference to the final Office Action and Advisory Action that were mailed in this case respectively on October 6, 2004 and August 11, 2005, submitted herewith is Appellants' Appeal Brief.

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Real Party in Interest

The real party in interest is The General Hospital Corporation, to whom all interest in the present application has been assigned (Reel 010520, Frame 0605).

Related Appeals and Interferences

There are no pending appeals or interferences related to this case.

Status of Claims

Claims 7, 10, 13-16, and 27-32 are currently pending.

Claims 7, 10, 13-16, and 27-32 were finally rejected in an Office Action mailed on October 6, 2004 and are appealed.

Claims 1-6, 8, 9, 11, 12, and 17-26 have been canceled.

Status of Amendments

All amendments filed in this application have been entered.

Summary of Claimed Subject Matter

Appellants' claimed subject matter is directed to a substantially pure RXR-interacting protein, including an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3) (See, for example, page 3, line 30 – page 4, line 5; and

page 7, line 2.) An exemplary protein includes an amino acid sequence that is at least 90% identical to the amino acid sequence of RIP15 shown in Figure 5 (SEQ ID NO: 3); is derived from a mammal such as a human; binds a β-retinoic acid response element (β-RARE) in the presence of RXR; or binds an ecdysone response element (EcRE) in the presence of RXR. (See, for example, page 3, line 30 – page 4, line 5; and page 7, line 3.) Typically, the protein includes an amino acid sequence that is at least 95% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3); an amino acid sequence that is identical to the amino acid sequence of RIP15 (SEQ ID NO: 3); or is the amino acid sequence of the protein identical to the amino acid sequence of RIP15 (SEQ ID NO: 3). (See, for example, page 3, line 30 – page 4, line 5; page 7, line 3; and Figure 5.) The invention also is directed to a protein that interacts with a retinoid X receptor in an *in vivo* interaction trap assay or a protein that inhibits retinoid X receptor-dependent activation of a β-RARE-linked nucleic acid. (See, for example, page 2, lines 27-29; page 3, line 30 – page 4, line 5; page 11, lines 8-13; and page 40, lines 8-19.)

In another aspect, Appellants' claimed invention is directed to a RXR-interacting protein produced by expression of a purified DNA encoding a protein including an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3). (See, for example, page 3, line 30 – page 4, line 5; page 7, line 2; and page 4, lines 18-20.)

Grounds of Rejection to be Reviewed on Appeal

This appeal presents five issues:

1. Whether the Office erred in rejecting claims 7, 10, 13-16, and 27-32 under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, for lack of utility.

2. Whether the Office erred in rejecting claims 7, 10, 13-14, 16, 27, 28, and 31-32 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification.

3. Whether the Office erred in rejecting claims 7, 10, 13-16, and 27-32 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

4. Whether the Office erred in finding that Appellants' priority application failed to provide adequate support under 35 U.S.C. § 112 for claims 7, 10, 13-14, 16, and 27-32.

5. And whether the Office erred in rejecting claims 7, 10, 13-14, 16, 27, 28, and 31-32 under 35 U.S.C. §§ 102(e) and 102(b), as being anticipated by Liao *et al.* (U.S.P.N. 5,639,616).

Argument

I. The Utility Rejection Should Be Reversed

Claims 7, 10, 13-16, and 27-32 were rejected under 35 U.S.C. § 101 and § 112, first paragraph, with the Examiner stating that the claimed invention is not supported by a substantial or specific asserted utility or by a well established utility that would enable

one skilled in the art to use the invention. The Office while referring to the Liao reference (U.S. Patent No. 5,369,616) does not rely upon any evidence in support of these rejections. Given the statements on utility of the claimed invention found in Appellants' specification, these rejections are therefore in error and should be reversed.

The Examiner maintains, for the reasons set forth in the Office action, of October 6, 2004 (p. 3), that

> [N]o evidence is provided that RIP-15 can inhibit thyroid hormone receptor in hyperthyroidism. Furthermore, no compounds which increase RIP-15 expression is taught in the specification. Applicants further argue that antibodies to RIP-15 can be used to detect or monitor RXR-related disease. However, no evidence has been provided that RIP-15 antibodies can be used to detect hyperthyroidism.
>
> ****
>
> The RXR does not provide nexus to the disease but the protein which binds RXR and RIP-15 is an orphan receptor whose function is not know and does not provide nexus to diseases merely based on interaction with RXR. In fact further experimentation is necessary to determine the function necessary to determine the function of RIP-15 and it's nexus to a disease.

As is discussed below, the Office has incorrectly applied the standard by which utility of an invention is evaluated and, therefore, each of the utility and enablement rejections should be reversed.

In addition, it is Appellants' understanding that, as required by the M.P.E.P. and case law, that the Office will either provide a rebuttal for each of Appellants' assertions of utility explained below or will reverse these rejections in view of the clarifications which have been provided during prosecution.

Appellants Assert Four Credible, Specific, and Substantial Utilities

The analysis to be carried out in making a rejection under 35 U.S.C. § 101 must include a determination of whether an assertion of utility has been made in an Appellants' specification and, if so, whether that asserted utility is credible (*i.e.*, whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided; M.P.E.P. § 2107.01-III(B)).

In the present case, Appellants assert the four utilities described below. Appellants submit that, absent data or evidence to the contrary provided by the Office, it is credible that administration of RIP15 protein or a compound that increases RIP15 expression will ameliorate RXR-associated conditions, and further that detection of decreased RIP15 levels in a subject using an anti-RIP15 antibody will identity subjects at increased risk for these conditions. Nonetheless, while the Office has stated that these utilities are not credible, no evidence has been provided that may be relied upon to reach this conclusion, as the Guidelines require. In particular, the Guidelines state that the Office

> must treat as true any statement of fact made by the Applicant in relation to the asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement... [I]t is improper to disregard the opinion [of a qualified expert] solely because of a disagreement over the significance or meaning of the facts offered. (M.P.E.P. § 2107, emphasis added)

To be properly rejected under § 101, the Guidelines set forth that a case must represent one of those rare instances that meets the stringent criterion of being "totally incapable of achieving a useful result," *Brooktree Corp. v. Advanced Micro Devices, Inc.*,

977 F.2d 1555, 1571, 24 USPQ 2d 1401, 1412 (Fed. Cir. 1992), (see M.P.E.P. § 2107.01-II). The only instances in which the federal courts have found a lack of patentable utility were where, "based upon the factual record of the case, it was clear that the invention could and did not work as the inventor claimed it did" (M.P.E.P. § 2107.01-II, emphasis added). These rare cases have been ones in which the appellant either (a) failed to disclose any utility for the invention, or (b) asserted a utility that could be true only "if it violated scientific principle, such as the second law of thermodynamics, or a law of nature, or was wholly inconsistent with contemporary knowledge in the art" (M.P.E.P. § 2107.02-IIIB).

Procedurally, the M.P.E.P. makes clear that the burden is on the Office to provide a detailed, reasoned explanation for the rejection that is supported, if possible, by documentary evidence indicating why the asserted utility is more likely than not "incredible." "An appellant's assertion of utility creates a presumption of utility" (M.P.E.P. § 2107.01-III(A)); "Where an appellant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed by Office personnel as being 'wrong,' even when there may be reason to believe that the assertion is not entirely accurate" (M.P.E.P. § 2107.01-III(B)). Conversely, if the Office determines that the claimed invention has a credible utility, neither a 35 U.S.C. § 101 nor a related 35 U.S.C. § 112 rejection may be applied (or, upon rebuttal of the Office's position, both rejections must be simultaneously reversed).

In the present case, Appellants, as discussed below, assert four utilities in the

specification, that are, on their face, credible. Appellants assert that the present invention provides RIP15 protein that can be used directly as a therapeutic, used to identify potential therapeutics that lead to decreased RXR activity, or used to generate diagnostic anti-RIP15 antibodies whereas, prior to the present invention, this was not possible because RIP15 was unavailable and its function was not known. At least some of the identified compounds that increase RIP15 expression are expected to have the proposed therapeutic activity of treating a RXR-associated disease (particularly hyperthyroidism). Additionally, one skilled in the art would appreciate that RIP15's ability to bind a β-RARE enables RIP15 to be used for the isolation or purification of a β-RARE from, for example, synthetic DNA libraries, genomic libraries, or cell lysates. The Office has provided no evidence to dispute any of these utilities, and on this basis alone the rejection should be reversed.

Standards for Satisfying the Utility Requirement

Appellants first note that the M.P.E.P. outlines the criteria to determine the utility of an invention. The utility of an invention must be specific and substantial or well-established. In defining the metes and bounds of a specific utility, the M.P.E.P. requires that:

> a utility [be] specific to the subject matter claimed. This contrasts with a general utility that would be applicable to the broad class of the invention ... A general statement of diagnostic utility, such as diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed (M.P.E.P. 2107.01 I, emphasis added)

By implication, therefore, the specific utility of a particular protein may be established by

the disclosure of a specific disease or condition with which it is associated.

Likewise, a substantial utility is established by a "real world" context of use, such as the identification of a material which has a correlation to, or impacts the onset or progression of a particular disease or condition. Specifically, the M.P.E.P. states:

> both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring (M.P.E.P. 2107.01 I; emphasis added).

Thus, a component of an assay method for identifying candidate compounds which may be used for treating a specific disease itself has substantial utility. Similarly, components of an assay method for measuring the presence of a material associated with a risk of disease have substantial utility.

Alternatively, the utility requirement of 35 U.S.C. § 101 can also be satisfied by identifying a well-established utility, which is defined in the Revised Interim Utility Guidelines Training Materials as

> A specific, substantial, and credible utility which is well known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art (page 7; emphasis added).

Of course, in evaluating the utility of the invention, the credibility of the disclosure must be assessed. Credibility must be viewed from the perspective of a person of ordinary skill in the art and should be based on the totality of the evidence (specification and prior

art) and reasoning provided.

The Federal Circuit in *In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995) has articulated the standard to be applied by the PTO in any challenge to an assertion of utility. In this case, the court stated:

> the PTO has the initial burden of challenging a presumptively correct assertion of utility in the disclosure. [citation omitted]. Only after the PTO provides evidence showing that one of ordinary skill in the art would reasonably doubt the asserted utility does the burden shift to the applicant to provide rebuttal evidence sufficient to convince such a person of the invention's asserted utility (page 1566; emphasis added).

The Office has failed to carry this burden. As discussed below, Appellants assert the specific and substantial utilities of using RIP15 (i) to inhibit RXR function in a subject for the treatment of an RXR-associated disease, such as hyperthyroidism, (ii) to identify a compound that increases RIP15 expression as a treatment for an RXR-associated disease (e.g., hyperthyroidism), and (iii) to generate an anti-RIP15 antibody for the detection or monitoring of an RXR-associated disease (e.g., hyperthyroidism). Further, the Office has presented no credible evidence that would cause a person of ordinary skill to doubt the asserted utilities of the present invention, much less the credibility of Appellants' disclosure. On these bases, this rejection should be reversed.

Specific Functions of RIP15

The present invention is based on Appellants' discovery of a novel receptor, RIP15, that interacts with the retinoid X receptor (RXR). The specificity of the interaction of RIP15 with RXR is demonstrated by the lack of interaction between RIP15 and other

nuclear receptors, such as TR, RAR, MB67, and GR (see, specification, for example, at page 16, Table 1). Additionally, heterodimers of RIP15 and RXR bind DNA specifically. In particular, RIP15 binds a β-RARE (β-retinoic acid response element) in the presence of RXR. Moreover, Appellants discovered that RIP15 completely blocks RXR-dependent transcription of a reporter gene linked to a β-RARE in a mammalian cell-based assay (see, specification, for example, at page 24, lines 7 to 24, and Figure 9). Thus, Appellants' specification demonstrates several important facts about RIP15 specificity that are the basis for Appellants' asserted utilities.

RIP15 Has Therapeutic Utility
For The Treatment of RXR-Associated Diseases

The disclosed ability of RIP15 to eliminate RXR-dependent activation of β-RARE linked genes strongly supports the specific utility of RIP15, analogs of RIP15, and fragments of RIP15 as therapeutics for the inhibition of RXR function in a subject (page 40, lines 8 to 19, and page 42). As stated on page 2, lines 14-24, of the specification:

> members of the RXR family play important roles in several aspects of development and central nervous system differentiation as well as in adult physiology. Based on both their specific response to the 9-cis-RA metabolite and their heterodimerization with the RARs, it is clear that the RXRs play a central role in the broad regulatory effects of retinoids. Moreover, their heterodimeric interactions with other family members indicate that the RXRs also play a central role in response to thyroid hormone, vitamin D, and perhaps other compounds.

From this disclosure, a skilled artisan would clearly understand that inhibiting RXR function is desirable for the treatment of diseases associated with an elevated level of hormone (*e.g.*, thyroid hormone, retinoic acid, or vitamin D) or hormone-mediated activity. For example,

hyperthyroidism is caused by the production of excess thyroid hormone, and thus hyperthyroidism can be treated by inhibiting the body's response to thyroid hormone. Because RXR is required for full hormone-dependent transcriptional activity of the thyroid hormone receptor-RXR complex, administration of RIP15 to a subject with hyperthyroidism would be expected to reduce the adverse effects caused by the excess thyroid hormone and the resulting excess thyroid hormone receptor activity (page 2, lines 4-6).

Appellants note that these asserted disease associations are neither general in nature, nor are they inconsistent with what one skilled in the art would expect for the specific disease involvement of RIP15 based on Appellants' disclosure of its ability to inhibit RXR function. Thus, Appellants have asserted a specific, substantial, and credible utility with a "real world" context for RIP15 proteins.

In the Advisory Action mailed August 11, 2005, the Examiner states

> Applicants argue that RIP 15 can be administered to inhibit hyperthyroidism because RIP15 would inhibit RXR interaction with thyroid hormone. However, if this was true then all RXR interacting protein such as vitamin D receptor can be used to treat hyperthyroidism and such treatment is not know [sic] to one skilled in the art although vitamin D receptor interaction with RXR has been known. Furthermore, no such treatment has been demonstrated with RIP15 and is proposed as a hypothetical treatment. Further experimentation is required and is not in readily available form because for example RIP15 is a nuclear receptor type protein and is found in the nucleaus [sic] of the cell and the mere administration intravenously will not result in the inhibition of the RXR if is [sic] occurs at all in situ.

These bases for rejecting Appellants' claims cannot be maintained as Appellants disclose RIP, analogs of RIP15, and fragments of RIP15 as having a substantial or practical

utility. Again, the Examiner's reasons are contrary to Federal Circuit case law and the M.P.E.P. More importantly, the Examiner provides no evidence to support the new reasons for rejection, or legitimate reasons why one skilled in the art would doubt Appellants' asserted utilities or disclosure.

Compounds that Increase RIP15 Expression Have Utility as Therapeutics for the Treatment of RXR-Associated Diseases

In addition to direct therapeutic use, RIP15 can also be used in standard methods to identify compounds that increase or decrease its expression and therefore its interaction with RXR (see, specification, for example, page 34, line 28 through page 35, line 12). One skilled in the art would appreciate that compounds that increase RIP15 expression are also useful for the treatment of diseases associated with an elevated level of hormone or hormone-mediated activity (e.g., hyperthyroidism). Again, the asserted utility of identifying compounds for the treatment of hyperthyroidism satisfies the criteria for a specific and substantial utility. The credibility of this utility is strongly supported by the disclosed "central role in response to thyroid hormone" of RXR and the reasonable conclusion that inhibiting RXR function is desirable for the treatment of diseases associated with elevated thyroid hormone levels or activity (page 2, line 23).

An Anti-RIP15 Antibody Has Utility for the Detection or Monitoring of RXR-Associated Diseases

RIP15 can also be used for the generation of anti-RIP15 antibodies for the detection or monitoring of RXR-related diseases (see, for example, page 40, line 20 through page 41,

line 12). For example, anti-RIP15 antibodies can be used to detect decreased levels of RIP15, which are likely associated with increased risk or severity of RXR-associated diseases such as hyperthyroidism. Again, the credibility of this specific and substantial utility is supported by Appellants' discovery of the ability of RIP15 to inhibit RXR function and the reasonable association of decreased RIP15 levels with increased RXR function. A skilled artisan would appreciate the high level of predictability between this increased RXR function and increased risk or severity of RXR-associated diseases such as hyperthyroidism. As the Examiner is aware, a compound (e.g., RIP15 protein) which enables the production of a useful end product (e.g., an anti-RIP15 antibody) is itself patentably useful under 35 U.S.C. § 101. *In re Kirk*, 376 F.2d 936 (C.C.P.A. 1967).

In the Advisory Action, the Examiner states:

> Applicants argue that antibody against RIP15 can be used to detect RIP15 associated disease. However, the specification does not teach the link between the amount of RIP15 and any specific diseases. Thus, the detection of the RIP15 level is not necessarily predictive of a diseas [sic] state. Furthermore, substantial experimentation is required because the detection with antibodies is not available in a readily usuable [sic] format where the nexus between RIP15 levels and disease state must be correlated. For example with regard to hyperthyroidism, the correlation is between thyroid hormone and the disease and the nexus to RXR level to disease has not been established much less the RIP15 interaction with RXR and thyroid hormone and hyperthyroidism. Thus, substantial experimentation is required use the antibody in detection of diseases.

These new bases for rejecting claim also do not find support in the case law or M.P.E.P. In addition, the Examiner attempts to challenge Appellants' disclosure without evidence. Nonetheless, the Examiner fails to address Appellants' central teaching that:

RIP15 inhibits RXR function. Moreover, the Examiner fails to address entirely the issue that Appellants' RIP15 protein itself enables the production of a useful end product (e.g., an anti-RIP15 antibody). See *In re Kirk*, 376 F.2d 936 (C.C.P.A. 1967).

RIP15 Has Utility for Purifying or Isolating β-RARE or β-RARE-Linked Nucleic Acids

In addition to the above three utilities, the specification also clearly conveys that RIP15 is able to bind a β-RARE, a known and useful material. For example, Sucov *et al.* (U.S.P.N. 5,091,518) reports that β-RAREs can be used to enhance transcriptional activity of promoters (abstract; column 1, lines 10-17; and column 2, lines 26-35). In particular, a β-RARE can be added to a vector encoding a protein of interest to generate an "enhanced expression system" that is responsive to retinoic acid (column 5, lines 48-50 and column 8, lines 30-41). The binding of RIP15 to β-RAREs allows RIP15 to be used to purify or isolate β-RAREs or β-RARE-linked nucleic acids. This utility would also have been apparent to one skilled in the art reading the specification, as binding of a β-RARE by RIP15 is discussed in the specification on pages 21 and 22. The use of RIP15 to isolate a β-RARE is sufficient to satisfy § 101. As noted above, RIP15 protein, which enables the production of a useful end product (e.g., purified β-RARE), is itself patentably useful under 35 U.S.C. § 101. *In re Kirk*, 376 F.2d 936 (C.C.P.A. 1967).[1]

Finally, in the Advisory Action, the Examiner states:

Applicants argue that RIP15 can be used to isolate B-RARE DNA promoter.

[1] The Office is also reminded that a patent is presumed valid under 35 U.S.C. § 282. Accordingly, one may presume that the utility disclosed in Sucov for the claimed β-RAREs is a valid, credible utility.

> However, the isolation does not have specific substantial utility because generally one skilled in the art isolate [sic] B-RARE by purification of already available bacterial host cell comprising the B-RARE in a vector and not by RIP15 binding isolation. Furthermore, there is no substantial utility because a nexus between the orphan receptor RIP15 and the use.

The Examiner, ignoring Appellants reliance on *In re Kirk*, 376 F.2d 936 (C.C.P.A. 1967), notably cites no case law in support of this requirement. Moreover, the Examiner does not challenge the clear teaching in Appellants' specification regarding the ability of RIP15 to eliminate RXR-dependent activation of β-RARE linked genes and that this teaching strongly supports the specific utility of RIP15, analogs of RIP15, and fragments of RIP15 as therapeutics for the inhibition of RXR function in a subject (page 40, lines 8 to 19, and page 42).

RIP15 Ligand is not Required for Asserted Utilities

In addition, contrary to the position taken by the Examiner, Appellants note that none of the four asserted utilities presented above require the identification of a ligand for RIP15. In particular, RIP15 and polypeptides derived from RIP15 can be tested for their ability to inhibit RXR in the cell-based assay described in Appellants' specification on pages 23 and 24 or in any animal model of disease without the use of a ligand for RIP15. A RIP15 ligand is also not needed to identify therapeutic compounds that modulate RIP15 expression, to generate anti-RIP15 antibodies for diagnostic applications, or to purify a β-RARE.

Summary

In sum, given the uses of RIP15 based on Appellants' demonstration of the ability of

RIP15 to interact with and inhibit RXR or to bind β-RAREs, the related rejections under 35 U.S.C. § 101 and § 112, first paragraph should be reversed. It is noted that all assertions must be shown to be incredible for this rejection to stand. For all of the above reasons, the claimed RXR-interacting proteins have both substantial and practical utilities. The burden is on the Office to provide a detailed, reasoned explanation for the rejection, and it is Appellants' understanding that the Office will either provide a rebuttal for each of Appellants' assertions of utility or will reverse these rejections in view of the clarifications that have been provided during prosecution.

II. The Written Description Rejection Should Be Reversed

Claims 7, 10, 13, 14, 16, 27, 28, and 31-32 were rejected under 35 U.S.C. § 112, first paragraph, for lack of a written description. This rejection should be withdrawn.

Independent claim 7 requires a substantially pure RXR-interacting protein that includes an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3). The other pending independent claim, claim 27, requires an RXR-interacting protein produced by expression of a purified DNA encoding a protein that includes an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3). Claims 10 and 28 require 90% identity and 95% identity, respectively, to SEQ ID NO: 3, and claim 32 requires that the protein inhibit retinoid X receptor-dependent activation of a β-RARE-linked nucleic acid.

The present rejection turns, in essence, on the assertion that the "essential feature" of the claimed invention is the RIP15 sequence of SEQ ID NO: 3. This rejection should be withdrawn.

Appellants again assert that 100% identity to the RIP15 sequence of SEQ ID NO: 3 is not essential to the present invention. In defining the term "RXR-interacting protein," the specification clearly teaches that proteins with at least 85% identity to RIP15 can also interact with RXR:

> By "RXR-interacting protein" is meant a polypeptide which directly or indirectly physically interacts with a retinoid X receptor in the in vivo protein interaction assay described herein.... Preferably, such a polypeptide has an amino acid sequence which is at least 85%, preferably 90%, and most preferably 95% or even 99% identical to the amino acid sequence of an interacting protein described herein (e.g., RIP14, RIP15, RIP110, or RIP13) at the point of interaction with the retinoid X receptor, or [is] at least 80% and preferably 90% identical overall.

(Specification, page 5, line 24 through page 6, line 5).

The specification further describes mutations that can be made to the RIP15 sequence to maintain the ability of the protein to interact with RXR. For example, page 6, line 26 through page 7, line 5 of the specification states:

> By "substantially identical" is meant an amino acid sequence which differs only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy the function of the protein (assayed, e.g., as described herein). Preferably, such a sequence is at least 85%, more preferably 90%, and most preferably 95% identical at the amino acid level to one of the sequences of Figs. 4, 5, 10, and 11 (SEQ ID NOS: 1-5).

Standard methods, such as those described on pages 41-43, can be used to generate proteins with at least 85% sequence identity to the disclosed sequence of RIP15 (SEQ ID NO: 3). These proteins are structurally characterized by this high level of sequence identity to SEQ ID NO: 3. As 100% sequence identity to SEQ ID NO: 3 is not necessary for the claimed invention, Appellants respectfully assert that it would be unfair to limit the present claims to only those proteins with 100% sequence identity to SEQ ID NO: 3.

Appellants further assert that one skilled in the art would appreciate that the essential feature of the claimed invention is the ability of the claimed proteins to interact with RXR. For example, page 3, line 30 through page 4, line 5 of the specification states:

> In a second aspect, the invention features a substantially pure preparation of a retinoid X receptor (RXR)-interacting protein. Preferably, the RXR-interacting protein is RIP14, RIP15, RIP110, or RIP13; or includes an amino acid sequence substantially identical to an amino acid sequence shown in any of Figs. 4, 5, 10, and 11 (SEQ ID NOS: 1-5); is derived from a mammal, for example, a human; binds a β-RARE site in the presence of RXR; or binds an EcRE site in the presence of RXR.

Appellants note that all of the pending claims, through the definition of "RXR-interacting protein," include the functional limitation that the protein interacts with RXR. Appellants further note that the *in vivo* interaction trap assay described in the specification can readily be used by one skilled in the art to determine whether a protein with at least 85% sequence identity to the sequence of RIP15 (SEQ ID NO: 3) interacts with RXR (see, for example, pages 11-14). Alternatively, a skilled artisan can easily determine whether the protein interacts with RXR by determining whether the protein inhibits RXR-dependent

activation of a β-RARE-linked nucleic acid (as disclosed, for example, on page 24, lines 7-24). Other standard methods for determining whether a protein interacts with RXR include gel filtration chromatography and co-immunoprecipitation assays.

In response to the Office's assertion that a functional limitation cannot be used to limit the claims because RIP15 is an orphan receptor, Appellants again respectfully assert that further characterization of RIP15, such as identification of a ligand for RIP15, is not necessary to distinguish the claimed proteins from other proteins. As stated in the M.P.E.P. (2163 II(A)3(a) (i)(c)(l)),

> [f]actors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient.

As noted above, the claimed proteins are distinguished from other proteins by both the structural characteristic of having at least 85% sequence identity to SEQ ID NO:3 and by the specific functional characteristic of interacting with RXR. In contrast, RIP15 does not bind other receptors, such as TR, RAR, MB67, and GR (page 16, Table 1). Additionally, Appellants note that the specification teaches other functional characteristics of RIP15. For example, RIP15 binds a β-RARE in the presence of RXR (claim 15) and inhibits RXR-dependent activation of a β-RARE-linked nucleic acid (claim 32). Based on Appellants' disclosure of these properties and routine assays for determining whether a particular protein

has these properties, one skilled in the art would appreciate that Appellants were in possession of the claimed invention.

As clear distinguishing characteristics that are shared by the claimed proteins are disclosed in Appellants' specification, this rejection should be reversed.

III. The Enablement Rejection Should Be Reversed

Claims 7, 10, 13-16, and 27-32 stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification fails to enable the skilled artisan to make and use the claimed invention. The Examiner asserts that the specification does not reasonably provide the full scope of enablement for RXR-interacting proteins comprisng an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO:3).

Claims 29 and 30

As an initial matter, Appellants point out that, with respect to claims 29 and 30, there can be no question that the enablement requirement is satisfied, as SEQ ID NO: 3 is presented in Appellants' specification. For this reason alone, as applied to claims 29 and 30, the § 112 rejection should be reversed.

Claims 7, 10, 13-16, 27-28, and 31-32

Appellants further note that undue experimentation is not required by the skilled artisan to make and use the claimed proteins. Sequence analysis software allows the rapid identification of a protein having an amino acid sequence that is 85% (or 90 or 95%) identical to SEQ ID NO: 3. Upon identification, such a protein may be readily tested for an

ability to specifically and physically interact with a retinoid X receptor (RXR) in an *in vivo* interaction trap assay using, for example, one of the protein interaction detection assays described in the specification at page 11, line 15, through page 15, line 12. In addition, the skilled artisan can routinely determine whether the protein interacts with RXR by determining whether the protein inhibits RXR-dependent activation of a β-RARE-linked nucleic acid (as disclosed, for example, on page 24, lines 7-24). Other routine methods for determining whether a protein interacts with RXR include gel filtration chromatography and co-immunoprecipitation assays. Moreover, the office has failed to provide any evidence that one skilled in the art would consider such screening as constituting undue experimentation.

None of these aforementioned methods constitute undue experimentation. Furthermore, Appellants provide examples demonstrating that the practice of this invention successfully identifies proteins having the desired characteristics. Appellants' specification has provided sufficient guidance and data to support the scope of the requested claims. Accordingly, Appellants respectfully submit that the skilled artisan would have absolutely no difficulty in determining whether any particular protein would fall into the scope of the claimed subject matter, or how to make and use such a protein. The enablement rejection has been applied in error and should be reversed.

IV. Appellants' Priority Application Supports The Pending Claims

The Examiner acknowledges Appellants claim for domestic priority under 35 U.S.C. § 120. Nonetheless, the Examiner maintains that "the provisional application upon which priority is claimed fails to provide adequate support under 35 U.S.C. § 112 for claims 7, 10, 13-14, 16, 27-32 of this application." Appellants disagree.

Application Serial No. 08/372,652 filed January 13, 1995 plainly provides adequate support for the presently claimed subject matter. The Summary of the Invention, Detailed Description of the Invention, and Example portions of the specification discuss thoroughly the claimed invention. For example, evidence from U.S. Patent Application Serial No. 08/372,652 specification itself supporting the present claims is as follows.

Claims	Exemplary Support in U.S.S.N. 08/372,652 filed January 13, 1995
7. (Previously presented) A substantially pure RXR-interacting protein, comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).	Claims 7 and 10, for example, as filed; and p. 7 (line 2).
10. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of RIP15 shown in Figure 5 (SEQ ID NO: 3).	Claims 7 and 10, for example, as filed; and p. 7 (line 3).
13. (Previously presented) The protein of claim 7, wherein said protein is derived from a mammal.	Claim 13, for example, as filed.
14. (Original) The protein of claim 13, wherein said mammal is a human.	Claim 14, for example, as filed.
15. (Previously presented) The protein of claim 13, wherein said protein binds a β-retinoic acid response element (β-RARE) in the presence of RXR.	For example, p. 4 (lines 3-4).
16. (Previously presented) The protein of claim 13, wherein said protein binds an ecdysone response element (EcRE) in the presence of RXR.	For example, p. 4 (lines 4-5).

27. (Previously presented) RXR-interacting protein produced by expression of a purified DNA encoding a protein comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).	Claims 27, 17, and 7 for example, as filed; p 3 (line 30) – p. 4 (line 5); and p. 7 (line 2); and p. 4 (lines 18-20).
28. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).	Claims 27, 17, and 7 for example, as filed; p 3 (line 30) – p. 4 (line 5); p. 7 (line 3).
29. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).	Claims 27, 17, 7, and 10, for example, as filed; p 9 (lines 14-15); and Figure 5.
30. (Previously presented) The protein of claim 7, wherein the amino acid sequence of said protein is identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).	For example, page 3 (line 30)- page 4 (line 2); claim 10 as filed; and Figure 5.
31. (Previously presented) The protein of claim 7, said protein interacting with a retinoid X receptor in an in vivo interaction trap assay.	For example, p. 2 (lines 27-29); and p. 11 (lines 8-13).
32. The protein of claim 7, said protein inhibiting retinoid X receptor-dependent activation of a β-RARE-linked nucleic acid.	For example, p. 40 (lines 8-19).

As U.S. Patent Application Serial No. 08/372,652 provides plain support for Appellants' claimed invention, Appellants are entitled to the priority date of this application.

V. The Novelty Rejections Should Be Reversed

Claims 7, 10, 13, 14, 16, 27, 28, and 32 were again rejected under 35 U.S.C. § 102(e) as being anticipated by Liao *et al.* (U.S.P.N. 5,639,616), a patent stemming from a continuation-in-part application, the parent of which had a filing date of November 10, 1993. Accordingly, the earliest possible § 102(e) date for this reference is

November 10, 1993.[2]

Appellants again direct the Examiner to the Declarations of inventor Dr. David Moore, filed December 28, 2001 and October 23, 2002 (copies provided herewith), presenting documentation that Appellants obtained an exemplary RIP15 cDNA sequence prior to November 10, 1993. Because the claimed invention was reduced to practice prior to the earliest filing date of Liao, Liao cannot constitute prior art to the present claims under 35 U.S.C. § 102(e). This rejection should therefore be withdrawn.

Appellants also note that the Examiner contends that

> The Declaration of Dr. Moore filed December 28, 2001 does not overcome the rejection because applicant did not provide [a] showing under 37 C.F.R. 1.608(b). See MPEP 2308.02.

Appellants note that they believe that these Declarations are entirely appropriate and this basis of the rejection should therefore be withdrawn. Should the rejection be maintained, Appellants again respectfully request that the Examiner provide a specific detailed reasoning for the stated requirement and conclusion.

Claims 7, 10, 13-14, 16, 27, 28, and 31-32 were also rejected under 35 U.S.C. § 102(b) as anticipated by Liao *et al.* (U.S.P.N. 5,639,616). As indicated above Appellants' present application claims priority to U.S. Application Ser. No. 08/372,652 (now U.S. Patent No. 5,932,699) filed January 13, 1995, which supports the present claims.

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[2] Appellants note that, because this reference is continuation-in-part application, the actual § 102(e) date may actually be the filing date of the continuation-in-part application, November 18, 1994.

Thus, Liao *et al.* (U.S.P.N. 5,639,616), which issued June 17, 1997, is not prior art to the claimed invention and this rejection should therefore be withdrawn.

Conclusion

Appellants respectfully request that the rejection of claims 7, 10, 13-16, and 27-32 be reversed and allowed.

Enclosed is a check for $250.00 in payment of the fee required by 37 C.F.R. § 41.20(b)(2) for filing this brief.

Appellant hereby petitions that the period for filing this brief in connection with the Notice of Appeal that was filed in connection with the above-captioned application on April 8, 2005 be extended for 5 months, to and including November 8, 2005. Enclosed is a check for $1,080.00 for the fee required by 37 C.F.R. § 1.17(a).

No additional fees are believed to be due at this time. If, however, there are any other charges, or any credits, in connection with filing this brief, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 8 November 2005

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Claims Appendix

1-6. (Canceled)

7. (Previously presented) A substantially pure RXR-interacting protein, comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).

8-9. (Canceled)

10. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of RIP15 shown in Figure 5 (SEQ ID NO: 3).

11-12. (Canceled)

13. (Previously presented) The protein of claim 7, wherein said protein is derived from a mammal.

14. (Original) The protein of claim 13, wherein said mammal is a human.

15. (Previously presented) The protein of claim 13, wherein said protein binds a β-retinoic acid response element (β-RARE) in the presence of RXR.

16. (Previously presented) The protein of claim 13, wherein said protein binds an ecdysone response element (EcRE) in the presence of RXR.

17-26. (Canceled)

27. (Previously presented) RXR-interacting protein produced by expression of a purified DNA encoding a protein comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).

28. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).

29. (Previously presented) The protein of claim 7, comprising an amino acid sequence that is identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).

30. (Previously presented) The protein of claim 7, wherein the amino acid sequence of said protein is identical to the amino acid sequence of RIP15 (SEQ ID NO: 3).

31. (Previously presented) The protein of claim 7, said protein interacting with a retinoid X receptor in an *in vivo* interaction trap assay.

32. (Previously presented) The protein of claim 7, said protein inhibiting retinoid X receptor-dependent activation of a β-RARE-linked nucleic acid.

Evidence Appendix

Bowie et al. (1990) Science 247:130710.....A

Sycou et al. (U.S.P.N. 5,091,518).....B

Liao et al. (U.S.P.N. 5,639,616) C

Declaration of Dr. David Moore filed December 28, 2001 under 37 C.F.R. § 1.131.....D
(This Declaration was entered into the record as noted in the Office Action
mailed January 5, 2004)

Declaration of Dr. David Moore filed October 23, 2002 under 37 C.F.R. § 1.131
(This Declaration was entered into the record as noted in the Office Action
mailed April 23, 2002).....E

Related Proceedings Appendix

None.

Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions

JAMES U. BOWIE,* JOHN F. REIDHAAR-OLSON, WENDELL A. LIM, ROBERT T. SAUER

An amino acid sequence encodes a message that determines the shape and function of a protein. This message is highly degenerate in that many different sequences can code for proteins with essentially the same structure and activity. Comparison of different sequences with similar messages can reveal key features of the code and improve understanding of how a protein folds and how it performs its function.

THE GENOME IS MANIFEST LARGELY IN THE SET OF PROteins that it encodes. It is the ability of these proteins to fold into unique three-dimensional structures that allows them to function and carry out the instructions of the genome. Thus, comprehending the rules that relate amino acid sequence to structure is fundamental to an understanding of biological processes. Because an amino acid sequence contains all of the information necessary to determine the structure of a protein (*1*), it should be possible to predict structure from sequence, and subsequently to infer detailed aspects of function from the structure. However, both problems are extremely complex, and it seems unlikely that either will be solved in an exact manner in the near future. It may be possible to obtain approximate solutions by using experimental data to simplify the problem. In this article, we describe how an analysis of allowed amino acid substitutions in proteins can be used to reduce the complexity of sequences and reveal important aspects of structure and function.

Methods for Studying Tolerance to Sequence Variation

There are two main approaches to studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. This method has been extremely powerful for proteins such as the globins or cytochromes, for which sequences from many different species are known (*2–7*). The second approach uses genetic methods to introduce amino acid changes at specific positions in a cloned gene and uses selections or screens to identify functional sequences. This approach has been used to great advantage for proteins that can be expressed in bacteria or yeast, where the appropriate genetic manipulations are possible (*3, 8–11*). The end results of both methods are lists of active sequences that can be compared and analyzed to identify sequence features that are essential for folding or function. If a particular property of a side chain, such as charge or size, is important at a given position, only side chains that have the required property will be allowed. Conversely, if the chemical identity of the side chain is unimportant, then many different substitutions will be permitted.

Studies in which these methods were used have revealed that proteins are surprisingly tolerant of amino acid substitutions (*2–4, 11*). For example, in studying the effects of approximately 1500 single amino acid substitutions at 142 positions in *lac* repressor, Miller and co-workers found that about one-half of all substitutions were phenotypically silent (*11*). At some positions, many different, nonconservative substitutions were allowed. Such residue positions play little or no role in structure and function. At other positions, no substitutions or only conservative substitutions were allowed. These residues are the most important for *lac* repressor activity.

What roles do invariant and conserved side chains play in proteins? Residues that are directly involved in protein functions such as binding or catalysis will certainly be among the most conserved. For example, replacing the Asp in the catalytic triad of trypsin with Asn results in a 10^4-fold reduction in activity (*12*). A similar loss of activity occurs in λ repressor when a DNA binding residue is changed from Asn to Asp (*13*). To carry out their function, however, these catalytic residues and binding residues must be precisely oriented in three dimensions. Consequently, mutations in residues that are required for structure formation or stability can also have dramatic effects on activity (*10, 14–16*). Hence, many of the residues that are conserved in sets of related sequences play structural roles.

Substitutions at Surface and Buried Positions

In their initial comparisons of the globin sequences, Perutz and co-workers found that most buried residues require nonpolar side chains, whereas few features of surface side chains are generally conserved (*6*). Similar results have been seen for a number of protein families (*2, 4, 5, 7, 17, 18*). An example of the sequence tolerance at surface versus buried sites can be seen in Fig. 1, which shows the allowed substitutions in λ repressor at residue positions that are near the dimer interface but distant from the DNA binding surface of the protein (*9*). These substitutions were identified by a functional

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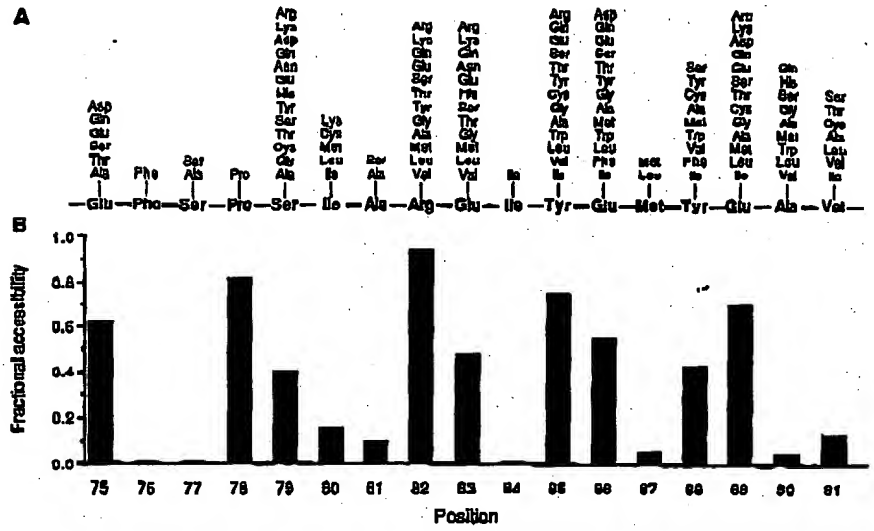

Fig. 1. (A) Amino acid substitutions allowed in a short region of λ repressor. The wild-type sequence is shown along the center line. The allowed substitutions shown above each position were identified by randomly mutating one to three codons at a time by using a cassette method and applying a functional selection (*9*). (B) The fractional solvent accessibility (*42*) of the wild-type side chain in the protein dimer (*43*) relative to the same atoms in an Ala-X-Ala model tripeptide.

selection after cassette mutagenesis. A histogram of side chain solvent accessibility in the crystal structure of the dimer is also shown in Fig. 1. At six positions, only the wild-type residue or relatively conservative substitutions are allowed. Five of these positions are buried in the protein. In contrast, most of the highly exposed positions tolerate a wide range of chemically different side chains, including hydrophilic and hydrophobic residues. Hence, it seems that most of the structural information in this region of the protein is carried by the residues that are solvent inaccessible.

Constraints on Core Sequences

Because core residue positions appear to be extremely important for protein folding or stability, we must understand the factors that dictate whether a given core sequence will be acceptable. In general, only hydrophobic or neutral residues are tolerated at buried sites in proteins, undoubtedly because of the large favorable contribution of the hydrophobic effect to protein stability (*19*). For example, Fig. 2 shows the results of genetic studies used to investigate the substitutions allowed at residue positions that form the hydrophobic core of the NH_2-terminal domain of λ repressor (*20*). The acceptable core sequences are composed almost exclusively of Ala, Cys, Thr, Val, Ile, Leu, Met, and Phe. The acceptability of many different residues at each core position presumably reflects the fact that the hydrophobic effect, unlike hydrogen bonding, does not depend on specific residue pairings. Although it is possible to imagine a hypothetical core structure that is stabilized exclusively by residues forming hydrogen bonds and salt bridges, such a core would probably be difficult to construct because hydrogen bonds require pairing of donors and acceptors in an exact geometry. Thus the repertoire of possible structures that use a polar core would probably be extremely limited (*21*). Polar and charged residues are occasionally found in the cores of proteins, but only at positions where their hydrogen bonding needs can be satisfied (*22*).

The cores of most proteins are quite closely packed (*23*), but some volume changes are acceptable. In λ repressor, the overall core volume of acceptable sequences can vary by about 10%. Changes at individual sites, however, can be considerably larger. For example, as shown in Fig. 2, both Phe and Ala are allowed at the same core position in the appropriate sequence contexts. Large volume changes at individual buried sites have also been observed in phylogenetic studies, where it has been noted that the size decreases and increases at interacting residues are not necessarily related in a simple complementary fashion (*5, 7, 17*). Rather, local volume changes are accommodated by conformational changes in nearby side chains and by a variety of backbone movements.

The Informational Importance of the Core

With occasional exceptions, the core must remain hydrophobic and maintain a reasonable packing density. However, since the core is composed of side chains that can assume only a limited number of conformations (*24*), efficient packing must be maintained without steric clashes. How important are hydrophobicity, volume, and steric complementarity in determining whether a given sequence can form an acceptable core? Each factor is essential in a physical sense, as a stable core is probably unable to tolerate unsatisfied hydrogen bonding groups, large holes, or steric overlaps (*25*). However, in an informational sense, these factors are not equivalent. For example, in experiments in which three core residues of λ repressor were mutated simultaneously, volume was a relatively unimportant informational constraint because three-quarters of all possible combinations of the 20 naturally occurring amino acids had volumes within the range tolerated in the core, and yet most of these sequences were unacceptable (*20*). In contrast, of the sequences that contained only

40	36	47	55	51	57	18
Ala	Ala	Ala	Ala	Cys	Cys	Ala
Cys	Cys	Cys	Ser	Val	Pro	Cys
Val	Thr	Thr	Cys	Ile	Val	Val
Ile	Val	Val	Thr	Leu	Ile	Ile
Leu	Ile	Ile	Val	Met	Leu	Leu
Met	Leu	Leu	Ile	Phe	Met	Met
Phe	Met	Met	Leu		Phe	
	Phe	Phe	Met			
			Phe			

Fig. 2. Amino acid substitutions allowed in the core of λ repressor. The wild-type side chains are shown pictorially in the approximate orientation seen in the crystal structure (*43*). The lists of allowed substitutions at each position are shown below the wild-type side chains. These substitutions were identified by randomly mutating one to four residues at a time by using a cassette method and applying a functional selection (*20*). Not all substitutions are allowed in every sequence background.

the appropriate hydrophobic residues, a significant fraction were acceptable. Hence, the hydrophobicity of a sequence contains more information about its potential acceptability in the core than does the total side chain volume. Steric compatibility was intermediate between volume and hydrophobicity in informational importance.

The Informational Importance of Surface Sites

We have noted that many surface sites can tolerate a wide variety of side chains, including hydrophilic and hydrophobic residues. This result might be taken to indicate that surface positions contain little structural information. However, Bashford *et al.*, in an extensive analysis of globin sequences (*4*), found a strong bias against large hydrophobic residues at many surface positions. At one level, this may reflect constraints imposed by protein solubility, because large patches of hydrophobic surface residues would presumably lead to aggregation. At a more fundamental level, protein folding requires a partitioning between surface and buried positions. Consequently, to achieve a unique native state without significant competition from other conformations, it may be important that some sites have a decided preference for exterior rather than interior positions. As a result, many surface sites can accept hydrophobic residues individually, but the surface as a whole can probably tolerate only a moderate number of hydrophobic side chains.

Identification of Residue Roles from Sets of Sequences

Often, a protein of interest is a member of a family of related sequences. What can we infer from the pattern of allowed substitutions at positions in sets of aligned sequences generated by genetic or phylogenetic methods? Residue positions that can accept a number of different side chains, including charged and highly polar residues, are almost certain to be on the protein surface. Residue positions that remain hydrophobic, whether variable or not, are likely to be buried within the structure. In Fig. 3, those residue positions in λ repressor that can accept hydrophilic side chains are shown in orange and those that cannot accept hydrophilic side chains are shown in green. The obligate hydrophobic positions define the core of the structure, whereas positions that can accept hydrophilic side chains define the surface.

Functionally important residues should be conserved in sets of active sequences, but it is not possible to decide whether a side chain is functionally or structurally important just because it is invariant or conserved. To make this distinction requires an independent assay of protein folding. The ability of a mutant protein to maintain a stably folded structure can often be measured by biophysical techniques, by susceptibility to intracellular proteolysis (*26*), or by binding to antibodies specific for the native structure (*27, 28*). In the latter cases, it is possible to screen proteins in mutated clones for the ability to fold even if these proteins are inactive. Sets of sequences that allow formation of a stable structure can then be compared to the sets that allow both folding and function, with the active site or binding residues being those that are variable in the set of stable proteins but invariant in the set of functional proteins. The DNA-binding residues of Arc repressor were identified by this method (*8*). The receptor-binding residues of human growth hormone were also identified by comparing the stabilities and activities of a set of mutant sequences (*28*). However, in this case, the mutants were generated as hybrid sequences between growth hormone and related hormones with different binding specificities.

Implications for Structure Prediction

At present, the only reliable method for predicting a low-resolution tertiary structure of a new protein is by identifying sequence similarity to a protein whose structure is already known (*29, 30*). However, it is often difficult to align sequences as the level of sequence similarity decreases, and it is sometimes impossible to detect statistically significant sequence similarity between distantly related proteins. Because the number of known sequences is far greater than the number of known structures, it would be advantageous to increase the reach of the available structural information by improving methods for detecting distant sequence relations and for subsequently aligning these sequences based on structural principles. In a normal homology search, the sequence database is scanned with a single test sequence, and every residue must be weighted equally. However, some residues are more important than others and should be weighted accordingly. Moreover, certain regions of the protein are more likely to contain gaps than others. Both kinds of information can be obtained from sequence sets, and several techniques have

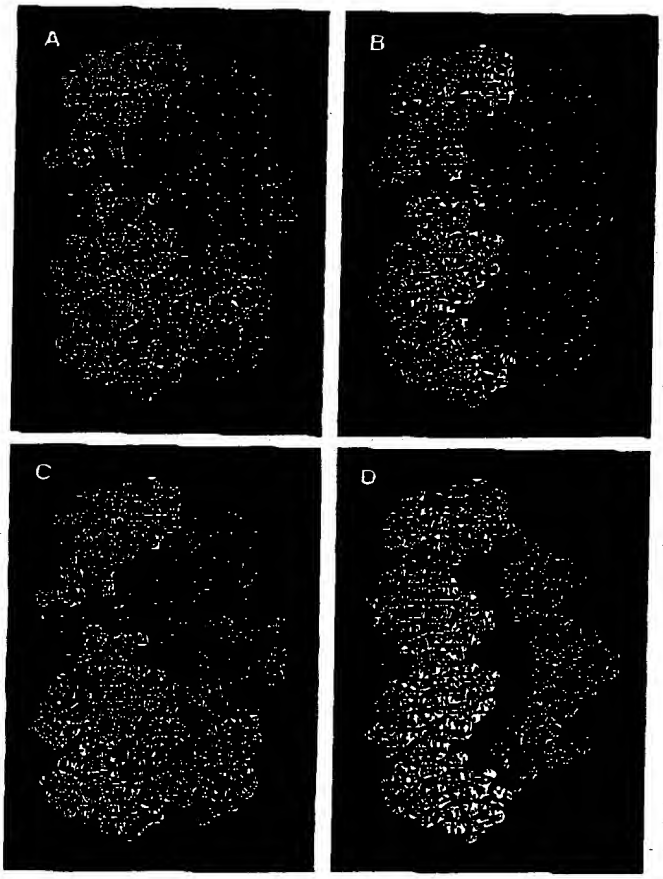

Fig. 3. Tolerance of positions in the NH_2-terminal domain of λ repressor to hydrophilic side chains. The complex (*45*) of the repressor dimer (blue) and operator DNA (white) is shown. In (A), positions that can tolerate hydrophilic side chains are shown in orange. The same side chains are shown in (B) without the remaining protein atoms. In (C), positions that require hydrophobic or neutral side chains are shown in green. These side chains are shown in (D) without the remaining protein atoms. About three-fourths of the 92 side chains in the NH_2-terminal domain are included in both (B) and (D). The remaining positions have not been tested. Data are from (*9, 14, 20, 27, 44*).

been used to combine such information into more appropriately weighted sequence searches and alignments (*31*). These methods were used to align the sequences of retroviral proteases with aspartic proteases, which in turn allowed construction of a three-dimensional model for the protease of human immunodeficiency virus type 1 (*29*). Comparison with the recently determined crystal structure of this protein revealed reasonable agreement in many areas of the predicted structure (*32*).

The structural information at most surface sites is highly degenerate. Except for functionally important residues, exterior positions seem to be important chiefly in maintaining a reasonably polar surface. The information contained in buried residues is also degenerate, the main requirement being that these residues remain hydrophobic. Thus, at its most basic level, the key structural message in an amino acid sequence may reside in its specific pattern of hydrophobic and hydrophilic residues. This is meant in an informational sense. Clearly, the precise structure and stability of a protein depends on a large number of detailed interactions. It is possible, however, that structural prediction at a more primitive level can be accomplished by concentrating on the most basic informational aspects of an amino acid sequence. For example, amphipathic patterns can be extracted from aligned sets of sequences and used, in some cases, to identify secondary structures.

If a region of secondary structure is packed against the hydrophobic core, a pattern of hydrophobic residues reflecting the periodicity of the secondary structure is expected (*33, 34*). These patterns can be obscured in individual sequences by hydrophobic residues on the protein surface. It is rare, however, for a surface position to remain hydrophobic over the course of evolution. Consequently, the amphipathic patterns expected for simple secondary structures can be much clearer in a set of related sequences (*6*). This principle is illustrated in Fig. 4, which shows helical hydrophobic moment plots for the Antennapedia homeodomain sequence (Fig. 4A) and for a composite sequence derived from a set of homologous homeodomain proteins (Fig. 4B) (*35*). The hydrophobic moment is a simple measure of the degree of amphipathic character of a sequence in a given secondary structure (*34*). The amphipathic character of the three α-helical regions in the Antennapedia protein (*36*) is clearly revealed only by the analysis of the combined set of homeodomain sequences. The secondary structure of Arc repressor, a small DNA-binding protein, was recently predicted by a similar method (*8*) and confirmed by nuclear magnetic resonance studies (*37*).

The specific pattern of hydrophobic and hydrophilic residues in an amino acid sequence must limit the number of different structures a given sequence can adopt and may indeed define its overall fold. If this is true, then the arrangement of hydrophobic and hydrophilic residues should be a characteristic feature of a particular fold. Sweet and Eisenberg have shown that the correlation of the pattern of hydrophobicity between two protein sequences is a good criterion for their structural relatedness (*38*). In addition, several studies indicate that patterns of obligatory hydrophobic positions identified from aligned sequences are distinctive features of sequences that adopt the same structure (*4, 29, 38, 39*). Thus, the order of hydrophobic and hydrophilic residues in a sequence may actually be sufficient information to determine the basic folding pattern of a protein sequence.

Although the pattern of sequence hydrophobicity may be a characteristic feature of a particular fold, it is not yet clear how such patterns could be used for prediction of structure de novo. It is important to understand how patterns in sequence space can be related to structures in conformation space. Lau and Dill have approached this problem by studying the properties of simple sequences composed only of H (hydrophobic) and P (polar) groups on two-dimensional lattices (*40*). An example of such a representation is shown in Fig. 5. Residues adjacent in the sequence must occupy adjacent squares on the lattice, and two residues cannot occupy the same space. Free energies of particular conformations are evaluated with a single term, an attraction of H groups. By considering chains of ten residues, an exhaustive conformational search for all 1024 possible sequences of H and P residues was possible. For longer sequences only a representative fraction of the allowed sequence or conformation space could be explored. The significant results were as follows: (i) not all sequences can fold into a "native" structure and only a few sequences form a unique native structure; (ii) the probability that a sequence will adopt a unique native structure increases with chain length; and (iii) the native states are compact, contain a hydrophobic core surrounded by polar residues, and contain significant secondary structure. Although the gap between these two-dimensional simulations and three-dimensional structures is large, the use of simple rules and sequence representations yields results similar to those expected for real proteins. Three-dimensional lattice methods are also beginning to be developed and evaluated (*41*).

Summary

There is more information in a set of related sequences than in a single sequence. A number of practical applications arise from an analysis of the tolerance of residue positions to change. First, such information permits the evaluation of a residue's importance to the function and stability of a protein. This ability to identify the essential elements of a protein sequence may improve our understanding of the determinants of protein folding and stability as well as protein function. Second, patterns of tolerance to amino acid substitutions of varying hydrophilicity can help to identify residues likely to be buried in a protein structure and those likely to occupy

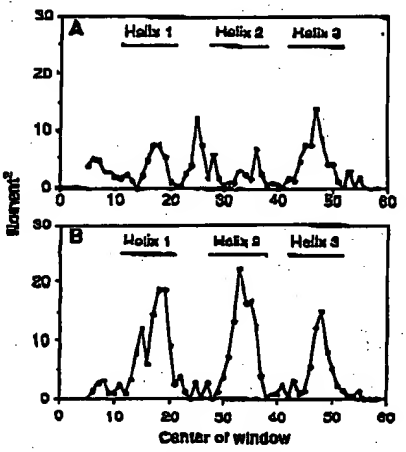

Fig. 4. Helical hydrophobic moments calculated by using (**A**) the Antennapedia homeodomain sequence or (**B**) a set of 59 aligned homeodomain sequences (*35*). The bars indicate the extent of the helical regions identified in nuclear magnetic resonance studies of the Antennapedia homeodomain (*36*). To determine hydrophobic moment, residues were assigned to one of three groups: H1 (high hydrophobicity = Trp, Ile, Phe, Leu, Met, Val, or Cys); H2 (medium hydrophobicity = Tyr, Pro, Ala, Thr, His, Gly, or Ser); and H3 (low hydrophobicity = Gln, Asn, Glu, Asp, Lys, or Arg). For the aligned homeodomain sequences, the residues at each position were sorted by their hydrophobicity by using the scale of Fauchere and Pliska (*45*). Arg and Lys were not counted unless no other residue was found at the position, because they contain long aliphatic side chains and can thereby substitute for nonpolar residues at some buried sites. To account for possible sequence errors and rare exceptions, the most hydrophilic residue allowed at each position was discarded unless it was observed twice. The second most hydrophilic residue was then chosen to represent the hydrophobicity of each position. An eight-residue window was used and the vectors projected radially every 100°. The vector magnitudes were assigned a value of 1, 0, or −1 for positions where the hydrophobicity group was H1, H2, or H3, respectively.

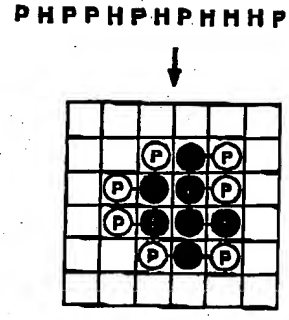

Fig. 5. A representation of one compact conformation for a particular sequence of H and P residues on a two-dimensional square lattice. [Adapted from (*40*), with permission of the American Chemical Society]

surface positions. The amphipathic patterns that emerge can be used to identify probable regions of secondary structure. Third, incorporating a knowledge of allowed substitutions can improve the ability to detect and align distantly related proteins because the essential residues can be given prominence in the alignment scoring.

As more sequences are determined, it becomes increasingly likely that a protein of interest is a member of a family of related sequences. If this is not the case, it is now possible to use genetic methods to generate lists of allowed amino acid substitutions. Consequently, at least in the short term, it may not be necessary to solve the folding problem for individual protein sequences. Instead, information from sequence sets could be used. Perhaps by simplifying sequence space through the identification of key residues, and by simplifying conformation space as in the lattice methods, it will be possible to develop algorithms to generate a limited number of trial structures. These trial structures could then, in turn, be evaluated by further experiments and more sophisticated energy calculations.

REFERENCES AND NOTES

1. C. J. Epstein, R. F. Goldberger, C. B. Anfinsen, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 439 (1963); C. B. Anfinsen, *Science* **181**, 223 (1973).
2. R. E. Dickerson, *Sci. Am.* **242**, 136 (March 1980).
3. M. D. Hampsey, G. Das, B. Sherman, *FEBS Lett.* **231**, 276 (1988).
4. D. Bashford, C. Chothia, A. M. Lesk, *J. Mol. Biol.* **196**, 199 (1987).
5. A. M. Lesk and C. Chothia, *ibid.* **136**, 225 (1980).
6. M. F. Perutz, J. C. Kendrew, H. C. Watson, *ibid.* **13**, 669 (1965).
7. C. Chothia and A. M. Lesk, *Cold Spring Harbor Symp. Quant. Biol.* **52**, 399 (1965).
8. J. U. Bowie and R. T. Sauer, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2152 (1989).
9. J. F. Reidhaar-Olson and R. T. Sauer, *Science* **241**, 53 (1988); *Proteins Struct. Funct. Genet.*, in press.
10. D. Shortle, *J. Biol. Chem.* **264**, 5315 (1989).
11. J. H. Miller *et al.*, *J. Mol. Biol.* **131**, 191 (1979).
12. S. Sprang *et al.*, *Science* **237**, 905 (1987); C. S. Craik, S. Roczniak, C. Largman, W. J. Rutter, *ibid.*, p. 909.
13. H. C. M. Nelson and R. T. Sauer, *J. Mol. Biol.* **192**, 27 (1986).
14. M. H. Hecht, J. M. Sturtevant, R. T. Sauer, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5685 (1984).
15. T. Alber, D. Sun, J. A. Nye, D. C. Muchmore, B. W. Matthews, *Biochemistry* **26**, 3754 (1987).
16. D. Shortle and A. K. Meeker, *Proteins Struct. Funct. Genet.* **1**, 81 (1986).
17. A. M. Lesk and C. Chothia, *J. Mol. Biol.* **160**, 325 (1982).
18. W. R. Taylor, *ibid.* **188**, 233 (1986).
19. W. Kauzmann, *Adv. Protein Chem.* **14**, 1 (1959); R. L. Baldwin, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8069 (1986).
20. W. A. Lim and R. T. Sauer, *Nature* **339**, 31 (1989); in preparation.
21. Lesk and Chothia (*5*) have argued that a protein core composed solely of hydrogen-bonded residues would also be inviable on evolutionary grounds, as a mutational change in one core residue would require compensating changes in any interacting residue or residues to maintain a stable structure.
22. T. M. Gray and B. W. Matthews, *J. Mol. Biol.* **175**, 75 (1984); E. N. Baker and R. E. Hubbard, *Prog. Biophys. Mol. Biol.* **44**, 97 (1984).
23. F. M. Richards, *J. Mol. Biol.* **82**, 1 (1974).
24. J. W. Ponder and F. M. Richards, *ibid.* **193**, 775 (1987).
25. J. T. Kellis, Jr., K. Nyberg, A. R. Fersht, *Biochemistry* **28**, 4914 (1989); W. S. Sandberg and T. C. Terwilliger, *Science* **245**, 54 (1989).
26. A. A. Pakula and R. T. Sauer, *Proteins Struct. Funct. Genet.* **5**, 202 (1989).
27. B. C. Cunningham and J. A. Wells, *Science* **244**, 1081 (1989); R. M. Breyer and R. T. Sauer, *J. Biol. Chem.* **264**, 13348 (1989).
28. B. C. Cunningham, P. Jhurani, P. Ng, J. A. Wells, *Science* **243**, 1330 (1989).
29. L. H. Pearl and W. R. Taylor, *Nature* **329**, 351 (1987).
30. W. J. Brown *et al.*, *J. Mol. Biol.* **42**, 65 (1969); J. Greer, *ibid.* **153**, 1027 (1981); J. M. Berg, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 99 (1988).
31. W. R. Taylor, *Protein Eng.* **2**, 77 (1988).
32. M. A. Navia *et al.*, *Nature* **337**, 615 (1989).
33. M. Schiffer and A. B. Edmundson, *Biophys. J.* **7**, 121 (1967); V. I. Lim, *J. Mol. Biol.* **88**, 857 (1974); *ibid.*, p. 873.
34. D. Eisenberg, R. M. Weiss, T. C. Terwilliger, *Nature* **299**, 371 (1982); D. Eisenberg, D. Schwarz, M. Komaromy, R. Wall, *J. Mol. Biol.* **179**, 125 (1984); D. Eisenberg, R. M. Weiss, T. C. Terwilliger, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 140 (1984).
35. T. R. Burglin, *Cell* **53**, 339 (1988).
36. G. Otting *et al.*, *EMBO J.* **7**, 4305 (1988).
37. J. N. Breg, R. Boelens, A. V. E. George, R. Kaptein, *Biochemistry* **28**, 9826 (1989); M. G. Zagorski, J. U. Bowie, A. K. Vershon, R. T. Sauer, D. J. Patel, *ibid.*, p. 9813.
38. R. M. Sweet and D. Eisenberg, *J. Mol. Biol.* **171**, 479 (1983).
39. J. U. Bowie, N. D. Clarke, C. O. Pabo, R. T. Sauer, *Proteins Struct. Funct. Genet.*, in preparation.
40. K. F. Lau and K. A. Dill, *Macromolecules* **22**, 3986 (1989).
41. A. Sikorski and J. Skolnick, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2668 (1989); A. Kolinski, J. Skolnick, R. Yaris, *Biopolymers* **26**, 937 (1987); D. G. Covell and R. L. Jernigan, *Biochemistry*, in press.
42. B. Lee and F. M. Richards, *J. Mol. Biol.* **55**, 379 (1971).
43. S. R. Jordan and C. O. Pabo, *Science* **242**, 893 (1988).
44. R. M. Breyer, thesis, Massachusetts Institute of Technology, Cambridge (1988).
45. J.-L. Fauchere and V. Pliska, *Eur. J. Med. Chem.-Chim. Ther.* **18**, 369 (1983).

46. We thank C. O. Pabo and S. Jordan for coordinates of the NH_2-terminal domain of λ repressor and its operator complex. We also thank P. Schimmel for the use of his graphics system and J. Bumbaum and C. Francklyn for assistance. Supported in part by NIH grant AI-15706 and predoctoral grants from NSF (J.R.-O.) and Howard Hughes Medical Institute (W.A.L.).

PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	David Moore et al.	Art Unit:	1646
Serial No.:	09/365,576	Examiner:	M. Pak
Filed:	August 2, 1999	Customer No.:	21559
Title:	RETINOID X RECEPTOR-INTERACTING POLYPEPTIDES AND RELATED MOLECULES AND METHODS		

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF DR. DAVID MOORE UNDER 37 C.F.R. § 1.131

I, David Moore, declare that:

1. I am an inventor of the invention described and claimed in the above-identified patent application.

2. The present claims of the application recite retinoid X receptor-interacting proteins that have an amino acid sequence that is at least 85% identical to that of RIP15.

3. The other inventors and I conceived of, and reduced to practice, the claimed subject matter of the present application prior to November 10, 1993.

4. The reduction to practice of the claimed invention is evidenced by Exhibits 1 and 2 annexed hereto. The dates of Exhibits 1 and 2 have been redacted in accordance with the standard practice, but are all prior to November 10, 1993. The experiments described and summarized in Exhibits 1 and 2 were performed by Wongi Seol, another inventor of the claimed invention. These experiments were carried out in the United States prior to November 10, 1993.

5. As described in the present application, we performed an *in vivo* interaction trap assay to isolate cDNA molecules encoding proteins that interact with the retinoid X receptor (RXR). For this assay, a mouse cDNA library was introduced into yeast that express a LexA-RXR fusion protein and that contain -galactosidase and LEU2 genes under the control of LexA binding sites. LexA-RXR is not a strong transcriptional activator in yeast. However, LexA-RXR activates expression from LexA binding sites in cells which also express a fusion protein consisting of a transcriptional activation domain joined to another protein which interacts specifically with RXR (as described on pages 12 and 25-27 of the specification).

Clone 15 which encodes RIP15 was isolated in this assay based on its ability to induce expression of both -galactosidase and LEU2, indicating that it encoded a protein that interacted with RXR.

6. Exhibit 1 is a laboratory notebook page that contains a picture of an agarose gel showing the analysis of the plasmid isolated from selected yeast clone 15 and transferred into *E. coli* for analysis (lower panel). The plasmid was purified from *E. coli* and digested with restriction enzymes to determine the size of the cDNA insert in the plasmid. Based on the migration of the restriction enzyme-digested cleavage product in the agarose gel, the cDNA insert was approximately 4.0 kDa (lower left corner of Exhibit 1). A region from this cDNA insert was used as a hybridization probe to isolate the full-length RIP15 coding sequence from a mouse cDNA library, as described on page 27 of the specification.

7. This full-length RIP15 cDNA was sequenced prior to November 10, 1993. Exhibit 2 contains exemplary RIP15 cDNA sequence. In particular, the sequence of primer ìp15 PCR 42merî which is listed in Exhibit 2 was designed based on the 5' terminus of this full-length RIP15 cDNA sequence. This primer was used to amplify the RIP15 sequence and add restriction sites flanking the RIP15 sequence to facilitate subsequent subcloning into an expression vector for the production of RIP15 protein. In this primer, the first nucleotide of the second RIP15 codon was altered to generate an NcoI restriction site and Kozak consensus nucleotides were included upstream of the start ìAUGî codon to maximize expression of RIP15 protein. The nucleotides from the RIP15 cDNA sequence that were included in this primer are 5'-CTTCCCCCACAAGTTCTCTG-3'.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 12/21/01

David Moore, Ph.D.
Professor
Molecular & Cellular Biology
Baylor College of Medicine

00786.246002 Declaration.wpd

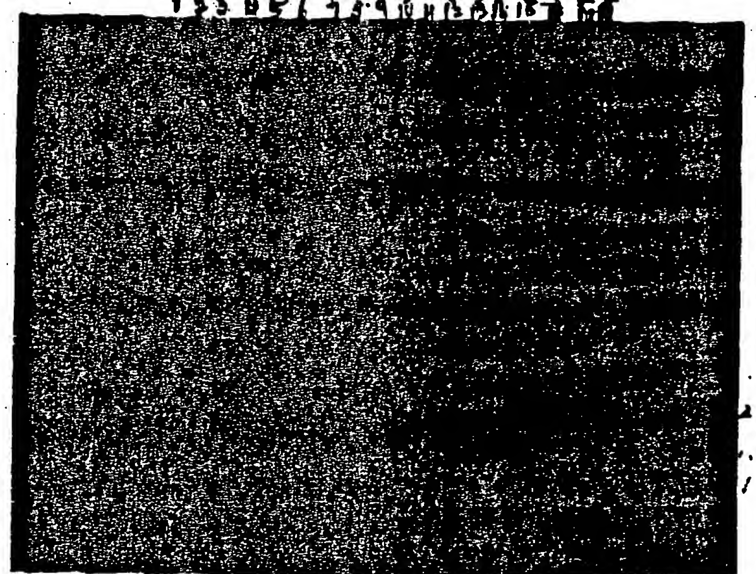
Exhibit

RXR - [illegible] screening

from (P3)
Plasmid isolation from E. coli MC1061/P3 transformant w/ No 1, 2, 5, 6, 7, 101-104, 106, 108, 109, C12 plus

each 3 colonies selected for mini prep → digestion w/ HincII

BstX2 2350-80
cgttrp2/HincII (950, 2500, 2600, 3000, 3330, 4700)
2.5/1.3/0.1+X, y + 0.12, 0.15, 0.35 (1-7)



marker ... Vcgttrp2)

HincII digestion

EcoRI 2370
BamHI 2985
BstX2 2350/80
RI/BamHI
cgtII 6.65/0.765
vector itself

(15)

→ Back to yeast transformation No1, No2(=3), No4(=5=6), No7(=8=9), No10(=11=12), No13(=14), No15, No16(=18), No17, No19(=20=21), No22 contam?, No23(=24), No25(=26=27), No28(=29), No31(=32=33), No34(=35)

EGY/SH 1Ex-RXR

No30 NO DNA

No36 ?

No1 vector, No2 RI/BamHI, No5, No6, No7, No101

No103, 104, 107 → just vector itself → forget it → no use

No1 - (1) ?
  - (2)=3 ?
No2 (4)=5=6 2.3kb
No5 (7)=8=9 1.9kb
No6 (10)=11 2.0
  - 12 1.4
No7 (13)=14 2.0
  - (15) ~4.0

→ 9423 → Blue

No101 (16)=(17)=18 ~2.0kb
No102 (19)-20=(21) ~1.4
  21 — incomplete digestion ? No diff. DNA
No105 (28)-29
  30 no DNA
No106 (31)-32=33 1.0kb
No108=104=107 → cgttrp2?
  see color?

4923 → Blue.

* 21 needs yeast

Exhibit 2

Brian:

Please make these oligomers. Thanks.

Wongi

p14 PCR 36mer

5' GCG CGC AAG CTT GCC ACC ATG GCC GCG GCA TCG

(AAG CTT = HindIII; ACC ATG GCC = NcoI.)

GCA

p15 PCR 42mer

5' GCG CGC AAG CTT GCC ACC ATG GCT TCC CCC ACA AGT

(AAG CTT = HindIII)

TCT CTG

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Commissioner for Patents
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DECLARATION OF DR. DAVID MOORE UNDER 37 C.F.R. § 1.131

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1. I am an inventor of the invention described and claimed in the above-identified patent application.

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3. The other inventors and I conceived of, and reduced to practice, the claimed subject matter of the present application prior to November 10, 1993.

4. The reduction to practice of the claimed invention is evidenced by Exhibit 1 annexed hereto. The date of Exhibit 1 has been redacted in accordance with the standard practice, but is prior to November 10, 1993. Exhibit 1 contains the entire amino acid sequence of RIP15, which was predicted based on the sequence of a full-length RIP15 cDNA. The isolation of the RIP15 cDNA was performed by Wongi Seol, another inventor of the claimed invention. The isolation and sequencing of the RIP15 cDNA was carried out in the United States prior to November 10, 1993.

5. As described in the present application and in the Declaration filed December 28, 2001, a RIP15 clone was isolated in an *in vivo* interaction trap assay designed to isolate cDNA molecules encoding proteins that interact with the retinoid X receptor (RXR). For this assay, a mouse cDNA library was introduced into yeast that expressed a LexA-RXR fusion protein and that contained β-galactosidase and LEU2 genes under the control of LexA binding sites. LexA-RXR is not a strong transcriptional activator in yeast. However, LexA-RXR activates expression from LexA binding sites in cells which also express a fusion protein consisting of a transcriptional activation domain joined to another protein which interacts specifically with RXR (as described on pages 12 and 25-27 of the specification).

Clone 15 which encodes RIP15 was isolated in this assay based on its ability to induce expression of both β-galactosidase and LEU2, indicating that it encoded a protein that interacted with RXR.

6. The plasmid was isolated from selected yeast clone 15 and transferred into

E. coli for analysis. In particular, the plasmid was purified from *E. coli* and digested with restriction enzymes to determine the size of the cDNA insert in the plasmid. Based on the migration of the restriction enzyme-digested cleavage product in the agarose gel, the cDNA insert was determined to be approximately 4.0 kDa. A region from this cDNA insert was used as a hybridization probe to isolate the full-length RIP15 coding sequence from a mouse cDNA library, as described on page 27 of the specification.

7. This full-length RIP15 cDNA was sequenced prior to November 10, 1993. Exhibit 1 contains the full-length translated amino acid sequence of RIP15.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 10/23/02

David Moore, Ph.D.
Professor
Molecular & Cellular Biology
Baylor College of Medicine

Exhibit 1

```
$ dir rxr*

Directory MOORE_LAB:[MOORE]

RXR14.;1            RXR14D.OUT;1        RXR14E.;2
RXR14E.OUT;3
RXR15.;2            RXR15E.;2           RXR15E.OUT;2
RXRG.;2

Total of 8 files.
$ ty rxr15.
rxr interacting clone 15   full length
   Rxr15.  Length: 446                          Type: P  Check:
7438  ..

       1  MSSPTSSLDT PVPGNGSPQP STSATSPTIK EEGQETDPPP GSEGSSSAYI

      51  VVILEPEDEP ERKRKKGPAP KMLGHELCRV CGDKASGFHY NVLSCEGCKG

     101  FFRRSVVHGG AGRYACRGSG TCQMDAFMRR KCQLCRLRKC KEAGMREQCV

     151  LSEEQIRKKR IQKQQQQQPP PPSEPAASSS GRPAASPGTS EASSQGSGEG

     201  EGIQLTAAQE LMIQQLVAAQ LQCNKRSFSD QPKVTPWPLG ADPQSRDARQ

     251  QRFAHFTELA IISVQEIVDF AKQVPGFLQL GREDQIALLK ASTIEIMLLE

     301  TARRYNHETE CITFLKDFTY SKDDFHRAGL QVEFINPIFE FSRAMRRLGL

     351  DDAEYALLIA INIFSADRPN VQEPSRVEAL QQPYVEALLS YTRIKRPQDH

     401  VRFPRMLMKL VSLRTLSSVH SEQVFALRLQ DKKLPPLLSE IWDVHE
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